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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	P03592US1
First Inventor or Application Identifier	WEAVER, Eric M.
Title	ANIMAL SERUM PRODUCT FOR GUT MUCOSAL PROTECTION AND PREVENTION OF INFECTION BY VEROTOXIN-PRODUCING ORGANISMS
Express Mail Label No.	EL193498359US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original and a duplicate for fee processing)*
2. [X] Specification [Total Pages 29] *(preferred arrangement set forth below)*
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [ ] Drawing(s) *(35 U.S.C. 113)* [Total Sheets]
4. Oath or Declaration [Total Pages]
   a. [X] Newly executed (original or copy)
   b. [ ] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 17 completed)* ***[Note Box 5 below]***
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).
5. [ ] Incorporation By Reference *(useable if Box 4b is checked)* The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. [ ] Microfiche Computer Program *(Appendix)*
7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
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   c. [ ] Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. [ ] Assignment Papers (cover sheet & document(s))
9. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [X] Power of Attorney
10. [ ] English Translation Document *(if applicable)*
11. [X] Information Disclosure Statement (IDS)/PTO-1449 [10] Copies of IDS Citations
12. [ ] Preliminary Amendment
13. [X] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
14. [X] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
15. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
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17. **If a CONTINUING APPLICATION,** *check appropriate box, and supply the requisite information below and in a preliminary amendment:*
    [ ] Continuation [ ] Divisional [X] Continuation-in-part (CIP) of prior application No: 09 / 210,940
    Prior application information: Examiner _____ Group / Art Unit: _____

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Applicant or Patentee: Eric M. Weaver
Serial No. or Patent No:
Filed or Issued:
For: ANIMAL SERUM PRODUCT FOR GUT MUCOSAL PROTECTION AND PREVENTION OF INFECTION BY VEROTOXIN-PRODUCING ORGANISMS

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27(c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

[ ] the owner of the small business concern identified below:
[X] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN LG Laboratories
ADDRESS OF CONCERN 2501 North Loop Drive, Suite 800, Ames, Iowa 50010

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled ANIMAL SERUM PRODUCT FOR GUT MUCOSAL PROTECTION AND PREVENTION OF INFECTION BY VEROTOXIN-PRODUCING ORGANISMS by inventor(s) Eric M. Weaver, described in

[X] the specification filed herewith.
[ ] application Serial No. _____, filed _____.
[ ] Patent No. _____, issued _____.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights in the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

*NOTE: *Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).*

FULL NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of payment, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME AND TITLE OF PERSON SIGNING Nix Lauridsen, President
ADDRESS OF PERSON SIGNING LG Laboratories, 2501 North Loop Drive, Suite 800, Ames, Iowa 50010
SIGNATURE _____ DATE 2/25/99

INVENTOR: **Eric M. Weaver**

TITLE: **ANIMAL SERUM PRODUCT FOR GUT MUCOSAL PROTECTION AND PREVENTION OF INFECTION BY VEROTOXIN-PRODUCING ORGANISMS**

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CROSS REFERENCE TO A RELATED APPLICATION

This application is a continuation-in-part of copending application Serial No. 09/210,490, filed December 11, 1998, the disclosure of which is hereby incorporated by reference.

FIELD OF THE INVENTION

This invention relates to a composition and method for prevention of disease. Specifically, this invention relates to the treatment of animals with an immunoglobulin product derived from animal serum to promote the gastrointestinal health of animals.

BACKGROUND OF THE INVENTION

Escherichia coli has long been associated with various types of disease. *E. coli* is a member of the coliform group of bacteria. As a normal inhabitant of the intestinal tract, *E. coli* are gram-negative, rod-shaped bacteria that are one of the most frequent causes of pyelonephritis and urinary tract infections. It is also an important cause of epidemic

diarrhea in nurseries for newborn infants. At least 137 serologic types of *E. coli* are known.

Recently, *E. coli* has reemerged as an important human pathogen because of the ability of certain strains to produce cytotoxins identified as shiga-like toxins (SLT). These cytotoxins are similar to shiga toxin, a toxin produced by *Shigella dysenteriae* type I, in both form and function. Shiga and shiga-like toxins are cytotoxic to vero cells, so are sometimes also referred to as verotoxins. For the purposes of this document, VT will herein be used as an acronym for verotoxin-producing organisms.

E. coli infection has traditionally been treated with antibiotics. The antibiotics of choice for typical *E. coli* infections include aminoglycosides, such as gentamicin and tobramycin, and third generation cephalosporins, such as ceftizoxime and ceftriaxone.

Verotoxin-producing *E. coli* (VTEC) strains are major bacterial pathogens that cause diarrhea in calves, edema disease in pigs, and have been implicated in diarrhea, hemorrhagic colitis, and the hemolytic-uremic syndrome in humans. One serotype, 0157:H7, is the dominant serotype associated with disease worldwide. However, other serotypes, such as 026:H11, 0103:H2, 0111:H-, and 113:H21, are also frequently implicated in disease. Numerous other serotypes of VTEC are either associated with disease at a low frequency or have not been implicated in disease. To date, more than 160 serotypes of VTEC have been identified among *E. Coli* strains isolated from human sources, and more than 200

serotypes have been isolated from cattle. Aleksic, S. (1995), "WHO report on Shiga-like toxin producing *Escherichia coli* (SLTEC), with an emphasis on zoonotic aspects." World Health Organization, Geneva, Switzerland.

E. coli 0157:H7, was first recognized as an important human pathogen in the United States in 1982, when the organism was identified as the cause of two geographically separate outbreaks of hemorrhagic colitis. Riley, Lee W. et al. (1983), "Hemorrhagic colitis associated with a rare *Escherichia coli* serotype," The New England Journal of Medicine, Vol. 308, 12:681-685. Outbreaks were often associated with the consumption of contaminated ground beef. However, the disease has also been associated with the consumption of ham, turkey, cheese sandwiches, potatoes, unpasteurized milk, apple cider, water, and vegetables. The hemorrhagic colitis is characterized by the sudden onset of severe abdominal cramps and grossly bloody diarrhea with little or no fever.

The pathogenesis of the enterohemorrhagic *E. coli* 0157:H7 strain is much different in comparison to other *E. coli* serotypes due to the deadly nature of the disease and the potency of the toxin. *E. coli* 0157:H7 produces verotoxins, that cause vascular lesions in several mammals, including humans. Only a very small inoculum of *E. coli* 0157:H7 is needed to produce enough toxin to cause disease. In fact, antibiotic therapy is not recommended for patients with *E. coli* 0157:H7 infection because the killing or disruption of the organisms may cause a greater release of

toxin and subsequent greater availability of toxin for absorption. Tarr, P.I. 1995. Escherichia coli 0157:H7: Clinical, Diagnostic, and Epidemiological Aspects of Human Infection. Clinical Infectious Diseases 20:1-10. The spectrum of illnesses associated with *E. coli* 0157:H7 infections ranges from asymptomatic infections to non-bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and death. Ryan, C.A. et al. (1986), "Escherichia coli 0157:H7 diarrhea in a nursing home: clinical, epidemiological, and pathological findings," Journal of Infectious Diseases, Vol. 154, 4:631-638.

Nearly 80% of the U.S. cattle population is infected with *E. coli* 0157:H7. Moon, H.W. Unpublished. However, mature cattle usually do not develop symptoms of disease. Since cattle are the natural reservoir of this particular serotype, bovine serum contains polyclonal, monospecific antibody against *E. coli* 0157:H7. The antibody component of serum consists of antitoxin to lipopolysaccharide(s) and SLT-I (shiga-like toxin I) and antibodies to the various cell components of the organism itself.

SLT-I is one of the toxins that are implicated as the cause of hemolytic uremic syndrome (HUS). HUS is characterized by rapid onset of acute hemolytic anemia, thrombocytopenia, and acute renal injury, usually after a prodromal illness of acute gastroenteritis with bloody diarrhea. The present inventor has discovered that normal bovine serum contains toxin neutralizing titer of >1:100 to SLT-I in a verocytotoxicity assay.

It has previously been demonstrated that beef colostrum contains antibodies which neutralize EHEC hemolysin, SLT-I and II in vitro. This antitoxin activity has been discussed in reference to the treatment of *E. coli* 0157:H7-induced diarrhea and in the prevention of hemolytic uremic syndrome. Further, Krivan et al. (U.S. Pat. No. 5,512,282) teach that administration of Shiga-toxin(s) to cattle produces neutralizing antibody to those toxins. However, these treatments fail to provide factors that inhibit colonization of *E. coli* 0157:H7 or other VTEC, thereby allowing the organism to continue to colonize and produce toxin. The level of toxin produced may then exceed the binding capacity of the antitoxin administered.

PCT/US97/20722 describes a method of treating HUS which includes the administration of a monoclonal antibody which binds specifically to Shiga-like toxin. The method again involves immunization of an animal and purifying the monospecific antibody component to the toxin(s).

Toxin-specific antibodies can be produced using well-recognized methods using mouse hybridoma or recombinant-DNA methods. These methods, however, are very expensive, thereby making them unaffordable to the majority of the world-wide population.

There is therefore a need in the art for a method of preventing and treating verotoxin-induced disease which is easy and less expensive to practice than other known treatments, which is also effective in binding the organisms

themselves, thereby preventing the continued release of toxin.

The present inventor has now discovered that polyclonal antibodies that are normally present in bovine serum provide sufficient enteric protection against disease from VTEC, including *E. coli* 0157:H7, as well as shiga-toxin-induced disease. The serum can be administrated to an animal orally to prevent infection and disease caused by VT.

Accordingly, it is a primary objective of the present invention to provide a composition and method for treating animals using a bovine serum composition enriched with antibodies directed to VT, including *E. coli* 0157:H7.

It is a further objective of the present invention to provide a composition and method for treating animals which is effective in reducing colonic verotoxin concentrations.

It is a further objective of the present invention to provide a composition and method for treating animals which reduces the number of VT organisms in the intestine and colon of the infected animal.

It is still a further objective of the present invention to provide a composition and method for treating animals which is convenient and economical to administer as an oral supplement.

It is still a further objective of the present invention to provide a composition and method for treating animals which is easy and economical to manufacture.

The method and means of accomplishing each of the above objectives as well as others will become apparent from the

detailed description of the invention which follows hereafter.

SUMMARY OF THE INVENTION

The invention describes an animal serum composition containing polyclonal, monospecific antibodies against Shigella, *E. coli* 0157:H7 and other VTEC which is administered to animals to protect their gastrointestinal health and prevent diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and edema disease in swine. The antibodies are specific to both the organism and verotoxins, including endotoxin, SLT-I, and SLT-II, and are effective in protecting the animal from hemorrhagic colitis, hemolytic uremic syndrome, and vascular disease.

The serum can be administered to the animal directly, or administered in a form which concentrates the naturally-occurring antibodies in the animal serum. The product is then administered to animals to improve their gastrointestinal health and prevent infection and subsequent disease from exposure to Shigella and VTEC organisms.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention relates to a new method and composition for preventing disease caused by verotoxin-producing organisms, including *E. coli* 0157:H7, and Shigella. The invention consists of a natural serum product which includes polyclonal, monospecific antibodies to both the organism and its toxins. The naturally-occurring antibodies can then be administered to animals to prevent infection

(disease) and neutralize toxin, thereby preventing subsequent disease.

As previously explained, cattle are the natural reservoir of *E. coli* 0157:H7. Because of this, they contain polyclonal, monospecific antibodies against this particular serotype, and its toxins. When an immunogenic substance is introduced into a living host, the host's immune system responds by producing antibodies to all of the recognizable sites on the substance. Many different antibody-producing cells from many different places in the host's body will be making antibodies to that part of the immunogenic substance by which they were activated; each antibody-producing clone making only one type of antibody to only one antigenic epitope on the foreign substance. This very broad response by the immunized host which results in the production of a broad range of antibodies of differing affinities and specificities for the immunogenic substance is called a polyclonal antibody response. The antibody component of bovine serum includes antitoxin to endotoxin, SLT-I (shiga-like toxin I), and antibody to the various components of the organism itself. SLT-II is weakly antigenic so anti-SLT-II titers are very low or undetectable in normal cattle sera. It is likely that porcine serum contains antitoxin to SLT-II.

The present invention is predicated upon the discovery that the administration of a bovine or other animal-type serum-derived product to animals is effective in preventing infection and subsequent disease from VT. The invention may be used to improve the growth and health of any animals,

including humans. For instance, the serum product is effective in preventing infection in humans due to food or water contaminated with Shigella, *E. coli* 0157:H7 and/or other VTEC. It is also effective in increasing weight gain and growth in pigs and other food animals. Once the threat of infection is removed, the animals consume more feed and water to improve weight gain and growth.

The invention also has applications in treating other types of animals, such as companion animals to reduce the incidence of disease and the subsequent likelihood of transmitting the disease to a human host. The serum product can also be administered to people to protect them from exposure to the organisms through typical *E. coli* 0157:H7 sources, such as contaminated food and water, swimming pools, apple cider, etc.

The polyclonal, monospecific antibodies may be administered as the serum isolated from whole blood. The serum source can be from any animal that has serum. In one embodiment of the invention the serum source is from the same species as that being treated. For purposes of convenience, the serum source is preferably bovine or porcine. The serum is then administered to an animal, preferably orally. The serum may also be administered via other routes including intravenously, intranasally, rectally, etc. The serum product may be administered as a tablet, through animal feed, or inexpensively through the animals' water.

Using a globulin separation method, the serum product may be further concentrated for administration as a globulin

concentrate. The globulin concentrate is stable in water, and therefore does not clog the lines of the animal's water system. Further, the globulin concentrate does not need to be placed in the food supply numerous times per day, thereby decreasing labor costs.

For purposes of human administration, the serum product or globulin concentrate may be administered through food, water, juices and other beverages, or milk products, such as yogurt. Such modes of administration are well known in the art, and have been used for such bacterial products as lactobacillus for many years.

The serum product or globulin concentrate can also be formulated into a pharmaceutical dosage form for oral administration, such as a tablet, capsule, suspension, granules, solution, etc. The pharmaceutical preparations of the present invention are manufactured in a manner which is itself well known in the art. The serum product or globulin concentrate is administered with a pharmaceutically acceptable carrier, which is herein defined as a carrier that is nontoxic to the animal, as well as compatible with the serum product or globulin concentrate.

For example the pharmaceutical preparations may be made by means of conventional mixing, granulating, dragee-making, dissolving, lyophilizing processes. The processes to be used will depend ultimately on the physical properties of the active ingredient used.

Suitable excipients are, in particular, fillers such as sugars for example, lactose or sucrose mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example, tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch, paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added, such as the above-mentioned starches as well as carboxymethyl starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are flow-regulating agents and lubricants, for example, such as silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate and/or polyethylene glycol. Dragee cores may be provided with suitable coatings which, if desired, may be resistant to gastric juices.

For this purpose concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate, dyestuffs and pigments may be added to the tablet of dragee coatings, for

example, for identification or in order to characterize different combination of compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. The serum or globulin protein fraction is placed in the carrier in a concentration that will deliver more than 2 mg immunoglobulin/kg bodyweight on a daily basis. The effective dose may vary according to the degree of bacterial challenge. For instance, higher levels of immunoglobulin may be necessary to treat an animal with an extreme bacterial challenge.

The serum product of the present invention can be administered to animals during all stages of their life cycles to prevent infection and disease from Shigella, *E. coli* 0157:H7, and other VTEC. Farmers and swine producers can use the serum product of the present invention to improve the health and growth rates of pigs and other food animals. Health management is critical in minimizing disease outbreaks

since such outbreaks are expensive in terms of medication cost and management time. This invention is successful in promoting the maintenance of enteric health by supplying an appropriate, adequate source of gastrointestinal protection to animals, including humans.

The serum product is also effective in protecting the gastrointestinal health of other animals, including horses and poultry. The opportunity for disease is decreased through the administration of the serum product.

The present inventor unexpectedly discovered that the serum product produced the beneficial results described above with a surprisingly low cytotoxin neutralization titer. As set forth in Example 1, the product reduced fecal verocytotoxicity in treated animals with a SLT-I neutralization titer of 1:8. As a result, humans and animals can be given a reasonable, low dose of material that will provide enteric protection against a challenge from VT-producing organisms.

The serum product of the present invention is derived from bovine or porcine serum, or other animal serum. The serum is obtained through conventional blood separation techniques. Typically, anticoagulant is first added to whole blood and then the blood is centrifuged to separate the plasma. Any anticoagulant may be used for this purpose, including sodium citrate and heparin. Persons skilled in the art can readily appreciate such anticoagulants. Calcium is then added to the plasma to promote clotting. This mixture is then centrifuged to remove the fibrin portion.

Once the fibrin is removed from plasma resulting in serum, the serum can be used as a principal source of immunoglobulins. Alternatively, one could also inactivate this portion of the clotting mechanism through the addition of various anticoagulants.

In addition, one could simply administer the plasma into the carrier as the immunoglobulin source. Either serum or plasma may be used as an immunoglobulin source in the globulin concentrate product. The further processing to concentrate immunoglobulin simply ensures fewer problems with compatibility of the concentrate with aqueous carriers and reduces the amount of material to be administered orally.

The serum may be further processed to increase the concentration of the immunoglobulin component. The defibrinated plasma is treated with an amount of salt compound or polymer sufficient to precipitate the albumin. Examples of phosphate compounds which may be used for this purpose include all polyphosphates, including sodium hexametaphosphate and potassium polyphosphate. The globulin may also be isolated through the addition of polyethylene glycol or ammonium sulfate. For reasons of convenience and economy, the polyphosphate compounds are preferably added to the plasma in a concentration of about 0.5-1% by weight of the plasma.

Following the addition of the phosphate compound, the pH of the plasma solution is lowered to a range of between 3.5-4.5 to stabilize the albumin precipitate. The pH should not be lowered below 3.5, as this will cause the proteins in the

plasma to become damaged. The preferred pH range is 3.5-4.0, with 3.95 being most preferred. Any type of acid can be used for this purpose, so long as it is compatible with the plasma solution. Persons skilled in the art can readily ascertain such acids. Examples of suitable acids are HCl, acetic acid, H_2SO_4, citric acid, and H_2PO_4. HCl is preferred, and 2N HCl is most preferred. The acid is added in an amount sufficient to lower the pH of the plasma to the designated range. Generally, this amount will range from a ratio of about 1:4 to 1:2 acid to plasma. The plasma is then centrifuged to separate the globulin fraction from the albumin fraction.

The next step in the process is to raise the pH of the globulin fraction with a base until it is no longer corrosive to separation equipment. Acceptable bases for this purpose include NaOH, KOH, and other alkaline bases. Such bases are readily ascertainable by those skilled in the art. NaOH is the preferred base, and a 10% solution of NaOH is most preferred. The pH of the globulin fraction is raised until it is within a non-corrosive range which will generally be between 5.0 and 9.0. The preferred pH range is 7.0-8.0, with 7.5 being most preferred. The immunoglobulin fraction is then preferably microfiltered to remove any bacteria that may be present.

The final immunoglobulin concentrate can optionally be spray-dried into a powder. The powder allows for easier packaging and the product remains stable for a longer period of time than the raw globulin concentrate in liquid or frozen form. The immunoglobulin concentrate powder has been found

to contain approximately 35-50% IgG. The immunoglobulin concentrate is then mixed with serum concentrate and compounds that improve wettability.

The immunoglobulin concentrate may then be prepared with one or more appropriate pharmaceutical excipients listed above into an oral dosage form for human or veterinary use.

The serum product may optionally be enriched with immunoglobulin from animals that have been previously exposed to a specific verotoxin-producing organism. The source of the immunoglobulin can be from any animal that has blood which includes immunoglobulins. For convenience, blood from beef, pork, and poultry processing plants is preferred. Enrichment of the natural immunoglobulin in the product increases the polyclonal, monospecific protection in the product. Typical levels of immunoglobulin from such serum provides adequate mucosal protection to prevent disease with a low-level exposure to the organism. Immunization or hyperimmunization of the animal is not necessary to provide temporary or even long-term protection to the animal. Serum collected from animals naturally exposed to the VT is tested to determine the lots with the highest antibody content to a specific organism. The supplemental immunoglobulin is placed in the composition in a concentration to achieve a significant daily intake of immunoglobulin, which is generall about 20 mg/kg body weight/day.

For purposes of administration to non-human animals, the serum product or globulin concentrate may be administered to the animal by placing it in the animal's water system via a

stock solution and a liquid dispenser. The globulin composition readily dissolves in water and remains stable in a highly-concentrated solution that does not obstruct the water line. While animals receive benefit from any amount of globulin composition placed in their water source, the concentration of globulin composition in the water should be at least 0.1% by weight. The response to the product is titratable, meaning a greater response is observed with a higher concentration so much higher levels of serum product or globulin concentrate can be added. The concentration of globulin can be increased until the water becomes saturated with globulin, i.e. the globulin can no longer be dispersed within the water. Various concentrations of stock solutions and/or injection rates may be used to alter the concentration of immunoglobulin in the water.

For economy and efficiency and to achieve best results, the globulin composition should be dispersed in the water in a concentration of from about 0.375 to about 3.0% by weight. The concentration of IgG in the water in this concentration ranges from approximately 0.1-0.75% by weight. A higher dose of IgG may be necessary if the animal is challenged with a large number of organisms or is infected and the product is being used for treatment purposes.

A preferred water dispenser for use with this invention is manufactured by Dosatron® and is sold as the Proportional Non Electric Liquid Dispenser. The dispenser is installed directly on the water supply line. The dispenser is activated by water pressure. As the water passes through the

dispenser it takes up the designated percentage of concentrate to deliver to the animals.

With respect to humans, the serum product can be administered through any of the aforementioned routes of administration in the same doses and concentrations cited above. For purposes of convenience, oral administration is preferred through such means as tablets or capsules, or as a supplement to milk products or water.

In general, the serum product should be administered in a concentration of from about 5-100 mg immunoglobulin/kg body weight to protect enteric health. The preferred concentration is from about 25 to 75 mg immunoglobulin/kg body weight. However, concentrations that will provide 375 mg immunoglobulin/kg body weight may be more effective in the event of infection.

The serum product may also be administered with certain additives or nutrients, such as carbohydrates, vitamins and minerals. The only requirement is that the additives also be compatible with the serum product or immunoglobulin concentrate. Such additives can be readily ascertained by those skilled in the art.

The following examples are offered to illustrate but not limit the invention. Thus, they are presented with the understanding that various formulation modifications as well as method of delivery modifications may be made and still be within the spirit of the invention.

EXAMPLE 1

Effect of Serum Product on Pigs Challenged with Shiga-Like Producing *E. coli* (STEC)

Bovine serum and porcine immunoglobulin concentrate were separated from bovine and porcine plasma, respectively, using the previously described procedures. Lactose, fructo-oligosaccharide, methionine, and potassium were then blended with the immunoglobulin sources to complete the composition. The composition of the final product is shown in Table 1:

Table 1

Ingredient Composition of Water-Stable Globulin Concentrate (% by weight)

Ingredient	As-is, %
Serum concentrate	52.21
Immunoglobulin concentrate	24.28
Lactose	15.00
Fructo-oligosaccharide	5.00
Potassium Chloride	1.66
Lecithin	1.00
DL-methionine	0.86
Total	100.00

The composition was found to contain about 61% by weight crude protein and about 21% by weight IgG.

The product mixture was then analyzed for antitoxin activity using an assay for verocytotoxin neutralization. In

powder form, the product contained by analysis a SLT-I neutralization titer of 1:8. This powder was then reconstituted with tap water and the pH reduced to approximately 4.5 with citric acid to produce a 3.7% w/w stock solution. The solution was used as the sole water source for the 11 pigs used in the trial. The control group of 4 pigs were provided with tap water. The seven challenged pigs were exposed to a reference VTEC strain, S1191. This reference strain produces the verotoxin SLT-II, the causitive agent of edema disease in swine. Three of the challenged pigs received the bovine serum-based concentrate, which was injected into the water line for a period of 7 days.

Verocytotoxicity was measured in the fecal material from the pigs at the end of the trial period. The results are shown in Table 2:

Table 2

Pig ID	Treatment	Verocytotoxicity	Gain, kg	ADG, g
6	1	4	6.33	352
16	1	16	6.79	377
17	1	8	5.90	328
18	1	16	6.59	366
2	2	4096	4.96	276
11	2	4096	3.49	194
13	2	4096	5.87	326
27	2	4096	1.95	108
1	3	16	6.84	380
12	3	8	7.00	389
25	3	4	7.04	391

*Treatment 1 = control group
Treatment 2 = challenged group
Treatment 3 = challenged group receiving bovine serum and porcine Ig concentrate

The average daily gain for the control group was 356 grams/day while the average daily gain for the challenged group that was not treated was only 226 grams/day. In contrast, the challenged group treated with the serum product of the present invention had an average daily gain of 387 grams/day. Thus, daily gain improved 9% in the treated animals versus the control group despite the VTEC challenge.

This study therefore demonstrates that the administration of the bovine/porcine serum product to animals is effective in the reduction of verotoxin concentrations in the colon and in preventing VTEC infection, even though the

verocytotoxicity neutralization titer was low. The oral administration of immunoglobulin is effective in the protection of gastrointestinal health.

EXAMPLE 2

Preferred Manufacturing Method For Globulin Concentrate

The following illustrates a preferred method of manufacturing the globulin concentrate of the present invention:

Bovine Plasma

↓

0.3% $CaCl_2$

↓

P-3000 Centrifuge to remove fibrin

↓

Allow any foam to dissipate → bovine serum
Filter sock 60 mesh

↓

Add 1% Hexameta Phosphate (granular form) by wt. to serum
This is made up as a stock solution
(1 lb to 1.7 lbs hot water) (≈ 230 lbs solution)
and added as a solution to the serum

↓

pH is adjusted to 3.9-3.96 range (3.95 is target),
with a 2N HCl solution
This is done as a slow time dependent addition
(approx 718.5 lbs/1000 gal)
After pH adjustment, allow 1 hour for reaction
completion while stirring

↓

Centrifuge Alpha Laval 717 feed rate approx 18 gal/min
To determine rate of feed and discharge, use microcentrifuge tubes to determine solids for discharge rate
(use 10,000 g x 10 min)

Wt. Supernatant
Wt. Liquid
Determine % solids

↙ ↘

Globulin Fraction	**Albumin Fraction**
This pH and form is stable for several days for SPC's and can be used to allow accumulation of material for spray dryer	This form is stable for several days so accumulation for the spray dryer is possible
↓	↓
Raise pH to 7.5 with 10% NaOH (≈ 186.3 lbs)- microfiltration to remove any bacteria	Dilute thick slurry by 50% with distilled water before processing Raise pH to 8.0 with 10% NaOH (≈ 116 lbs) slowly - Do not burn proteins Microfiltration to remove any bacteria
↓	↓
Dialyze 20,000 molec. wt. membranes to conductivity of 1.5 ms/cm at 12g/dl protein	Dialyze (20,000 m.w. membranes) to conduction of 1.5 ms/cm at 12 g/dl protein level
↓	↓
Concentrate and spray dry 294°C inlet and 95°C outlet	Concentrate and spray dry 294°C inlet and 95°C outlet
↓	↓
Analyze spray dried powder	Analyze spray dried powder

Total Protein	
Albumin	Phospholipids
IgG	Phosphorus
Chol	Sodium
Trig	Chloride
Verotoxin-neutralization titer	Potassium

Total Protein	
Albumin	Phosphorus
IgG	Calcium
Chol.	Sodium
Trig	Chloride
Phoslip	Potassium

Having described the invention with reference to particular compositions, theories of effectiveness, and the like, it will be apparent to those of skill in the art that it is not intended that the invention be limited by such illustrative embodiments or mechanisms, and that modifications can be made without departing from the scope or spirit of the invention, as defined by the appended claims. It is intended that all such obvious modifications and variations be included within the scope of the present invention as defined in the appended claims. The claims are meant to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates to the contrary.

What is claimed is:

1.

A composition for promoting gastrointestinal health and prevention of infection by verotoxin-producing bacteria in animals comprising:
polyclonal, monospecific antibodies isolated from animal plasma.

2.

A composition according to claim 1 further including a pharmaceutically acceptable carrier.

3.

A composition according to claim 1 that is formulated into a dosage form selected from the group consisting of tablet, capsule, solution, granules, powder, and suspension for internal administration.

4.

A composition according to claim 1 containing at least 15% IgG by weight.

5.

A composition according to claim 2 wherein the monospecific antibodies are present in a concentration to achieve a verotoxin neutralization titer of equal to or greater than 1:8.

6.

A composition according to claim 1 which is processed to increase the total globulin content.

7.

A composition according to claim 6 which contains two to four fold more globulin protein than untreated bovine serum.

8.

A composition according to claim 1 further including: supplemental polyclonal monospecific antibodies from an animal, wherein the animal has been previously exposed to a verotoxin producing organism.

9.

A composition according to claim 1 further including: supplemental polyclonal monospecific antibodies from an animal, wherein the animal has been previously exposed to a bacterial toxin selected from the group consisting of shiga-toxin and shiga-like toxin.

10.

A method of preventing infection and disease from verotoxin-producing bacteria in animals comprising:
internally administering a composition to an animal which comprises polyclonal, monospecific antibodies isolated from natural animal serum.

11.

A method according to claim 10 wherein the animal serum source is from the same species of animal that the composition is administered to.

12.

A method according to claim 10 wherein the animal serum source is selected from the group consisting of bovine and porcine.

13.

A method according to claim 10 wherein the composition is administered in a dose of from about 1 g to about 30 g of plasma protein per day.

14.

A method according to claim 10 wherein the composition is administered through the animal's feed or water.

15.

A method according to claim 10 wherein the composition is administered in a dosage form selected from the group consisting of tablets, capsules, granules, liquid, suspension, and powder.

16.

A method according to claim 10 wherein the composition is administered in dairy products.

17.

A method according to claim 10 wherein the composition is administered orally.

18.

A method of manufacturing a composition for preventing infection and disease in animals from verotoxin-producing bacteria comprising:

obtaining blood from an animal source, wherein the blood includes a plasma and a red cell fraction;

separating the plasma from the red cell fraction;

removing the fibrin from the plasma to form serum;

removing a portion of the albumin from the serum to form concentrated globulin protein fraction; and

placing the globulin protein fraction in a pharmaceutically acceptable carrier.

19.

A method according to claim 18 wherein the globulin protein fraction is placed in the carrier in a concentration of from about 25 to about 95% by weight.

20.

A method according to claim 18 wherein supplemental immunoglobulin is placed in the composition.

ABSTRACT OF THE DISCLOSURE

A method and composition for preventing infection and disease from verotoxin-producing bacteria is described. The invention includes polyclonal, monospecific antibodies concentrated from animal serum. The immunoglobulin fraction from natural animal serum may be combined with antibodies to EHEC-specific colonization factors from an animal different from the source of the serum. The composition is effective in protecting gastrointestinal health and preventing diarrhea, hemorrhagic colitis and hemolytic uremic syndrome.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

COMBINED DECLARATION AND POWER OF ATTORNEY

FOR SOLE INVENTOR (C-I-P APPLICATION)

As the below named inventor I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor, of the subject matter which is claimed and for which a patent is sought on the invention entitled as follows: **ANIMAL SERUM PRODUCT FOR GUT MUCOSAL PROTECTION AND PREVENTION OF INFECTION BY VEROTOXIN-PRODUCING ORGANISMS**, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code Of Federal Regulations, Section 1.56. I further declare that no application for patent or inventor's certificate on this invention has been filed by me, my legal representative or assigns in any country foreign to the United States of America except as identified below:

NONE.

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112.

I acknowledge the duty to disclose information that is material to the examination of this application, namely, information where there is substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS
DESIGNATING THE U.S. FOR BENEFIT
UNDER 35 U.S.C. § 120:

U.S. APPLICATIONS Status (Check One)

Appln. No.	Filing Date		Patented	Pending	Abandoned
09/210,490	12/11/98			X	

PCT APPLNS. DESIGNATING THE U.S.

PCT Appln. No.	PCT Filing Date	U.S. Serial No. Assigned (if any)	Patented	Pending	Abandoned

And I hereby appoint ZARLEY, McKEE, THOMTE, VOORHEES & SEASE, comprising Donald H. Zarley, Registration No. 18,543; Bruce W. McKee, Registration No. 19,651; Dennis L. Thomte, Registration No. 22,497; Michael G. Voorhees, Registration No. 25,715; Edmund J. Sease, Registration No. 24,741; Mark D. Hansing, Registration No. 30,643; Kirk M. Hartung, Registration No. 31,021; Mark D. Frederiksen, Registration No. 31,357; Daniel J. Cosgrove, Registration No. 36,770; Michael R. Crabb, Registration No. 37,298; Heidi Sease Nebel, Registration No. 37,719; Wendy K. Marsh, Registration No. 39,705; and Jeffrey D. Harty, Registration No. 40,639; 801 Grand Avenue, Suite 3200, Des Moines, Iowa 50309, Telephone 515-288-3667, as my attorneys to prosecute this application and to transact all business in the Patent Office connected therewith.

I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE

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This declaration ends with this page.